

Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651

Confirmation No.: 9704

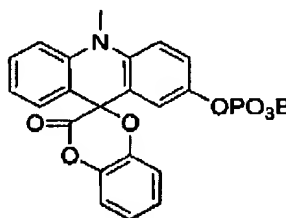
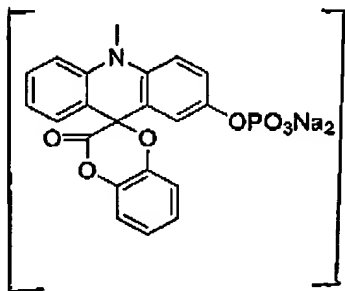
AMENDMENT TO THE CLAIMS

1-7. (Cancelled)

8. (Currently amended) The chemiluminescent substrate of claim 43 wherein said counter ions ~~are~~ A⁻ is selected from the group consisting of CH_3SO_4^- , FSO_3^- , CF_3SO_3^- , $\text{C}_4\text{F}_9\text{SO}_3^-$, $\text{CH}_3\text{C}_6\text{H}_4\text{SO}_3^-$, halide, CF_3COO^- , CH_3COO^- , and NO_3^- .

9-21. (Cancelled)

22. (Currently amended) The chemiluminescent substrate of claim 61 having the following structure:



23-24. (Cancelled)

25. (Cancelled)

26-28. (Cancelled)

29. (Cancelled)

Application No. 09/626,566

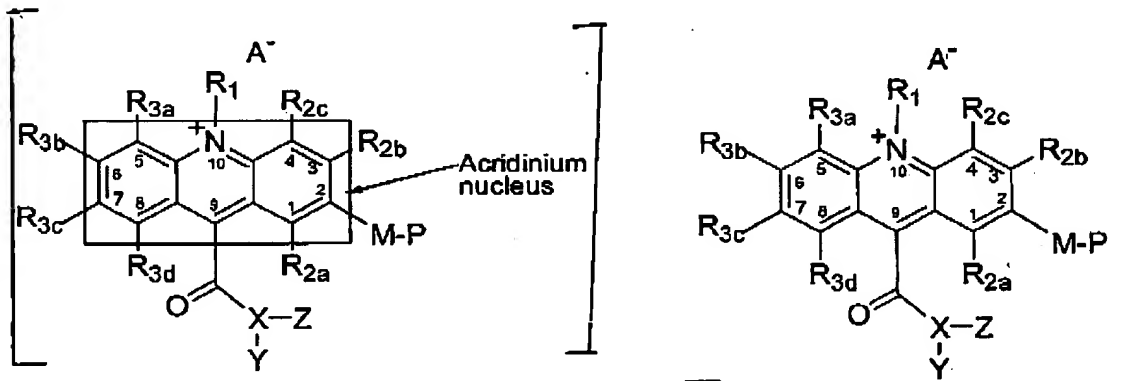
Filed: July 27, 2000

Group Art Unit: 1651

Confirmation No.: 9704

30-42. (Cancelled)

43. (Currently amended) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



wherein

P is PO_3Na_2 — PO_3B or a sugar moiety and B is a divalent cation or two monovalent cations selected from the group consisting of Na_2 , H_2 , K_2 , Ca and Mg;

M is oxygen;

R_1 is selected from the group consisting of methyl, sulfopropyl and sulfobutyl;

R_{2a} , R_{2b} , R_{2c} , R_{3a} , R_{3b} , R_{3c} and R_{3d} are hydrogen;

A^- is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A^- not being present if said R_1 substituent contains a strongly ionizable group

Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651

Confirmation No.: 9704

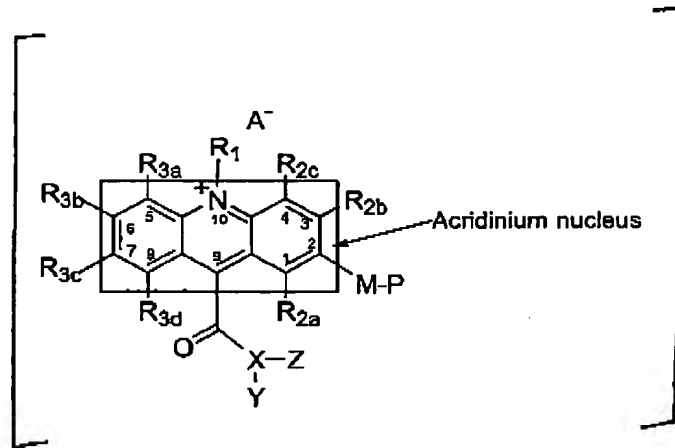
that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N ~~or~~ and S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, and (2',6'-dimethyl-4'-carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

44. (Currently amended) A The chemiluminescent substrate of claim 43, wherein a hydrolytic enzyme, said substrate having the structure,



wherein

P is PO₃B ~~PO₃Na₂~~ or a sugar moiety;

Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651

Confirmation No.: 9704

~~M is oxygen;~~

~~R₁ is selected from the group consisting of methyl,
sulfopropyl and sulfobutyl;~~

~~R_{2a}, R_{2b}, R_{2c}, R_{3a}, R_{3b}, R_{3c} and R_{3d} are hydrogen;~~

~~A⁻ is a counter ion for the electroneutrality of the
quaternary nitrogen of the acridinium compounds, said A⁻ not being
present if said R₁ substituent contains a strongly ionizable group
that can form an anion and pair with the quaternary ammonium
cationic moiety; and~~

X is O; Y is selected from the group consisting of phenyl,
(2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, and (2',6'-dimethyl-
4'-carboxyl)phenyl; and Z is omitted.

45. (Currently amended) The chemiluminescent substrate of claim
43, wherein

P is $\text{PO}_3\text{Na}_2\text{PO}_3\text{B}$;

X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

46. (Currently amended) The chemiluminescent substrate of claim
43, wherein

P is $\text{PO}_3\text{Na}_2\text{PO}_3\text{B}$;

Application No. 09/626,566

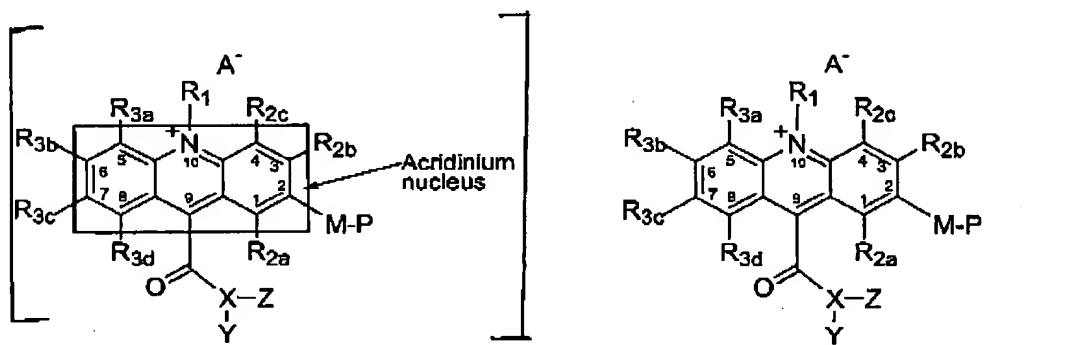
Filed: July 27, 2000

Group Art Unit: 1651

Confirmation No.: 9704

X is S; Y is selected from the group consisting of phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, and (2',6'-dimethyl-4'-carboxyl)phenyl; and Z is omitted.

47. (Currently amended) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



wherein

P is PO_3Na_2 , PO_3B or a sugar moiety and B is a divalent cation or two monovalent cations selected from the group consisting of Na_2 , H_2 , K_2 , Ca and Mg ;

M is oxygen;

R_1 is selected from the group consisting of methyl, sulfoalkyl and carboxymethyl;

R_{2a} , R_{2b} , R_{2c} , R_{3a} , R_{3b} , R_{3c} and R_{3d} can be the same or different, and are selected from the group consisting of hydrogen, methyl, methoxy, halides, and cyano ($-\text{CN}$);

Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651

Confirmation No.: 9704

A⁻ is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A⁻ not being present if said R₁ substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N ~~or~~ and S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl and Y is carboxypropyl.

48. (Currently amended) The chemiluminescent substrate of claim 47 wherein said counter ~~ions-ion~~ A⁻ ~~are-is~~ selected from the group consisting of CH₃SO₄⁻, FSO₃⁻, CF₃SO₃⁻, C₄F₉SO₃⁻, CH₃C₆H₄SO₃⁻, halide, CF₃COO⁻, CH₃COO⁻ and NO₃⁻.

Application No. 09/626,566

Filed: July 27, 2000

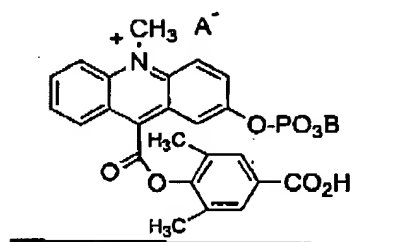
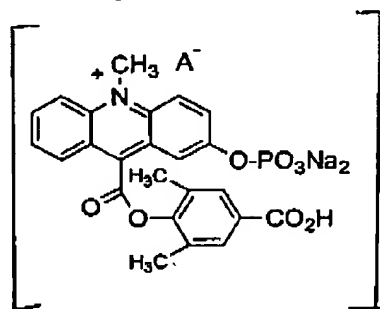
Group Art Unit: 1651

Confirmation No.: 9704

49. (Cancelled)

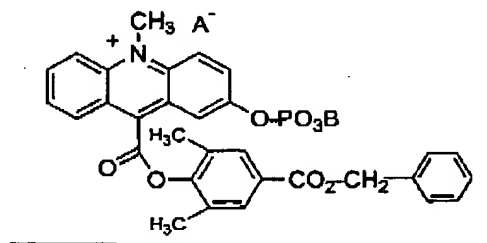
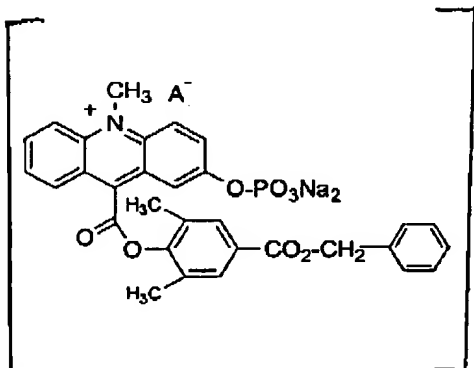
50. (Cancelled)

51. (Currently amended) The chemiluminescent substrate of ~~Claim~~
claim 43 -having the structure-



wherein A⁻ is selected from the group consisting of CH₃SO₄⁻, FSO₃⁻,
 CF₃SO₃⁻, C₄F₉SO₃⁻, CH₃C₆H₄SO₃⁻, halide, CF₃COO⁻, CH₃COO⁻ and NO₃⁻.

52. (Currently amended) The chemiluminescent substrate of ~~Claim~~
claim 43 having the structure-



Application No. 09/626,566

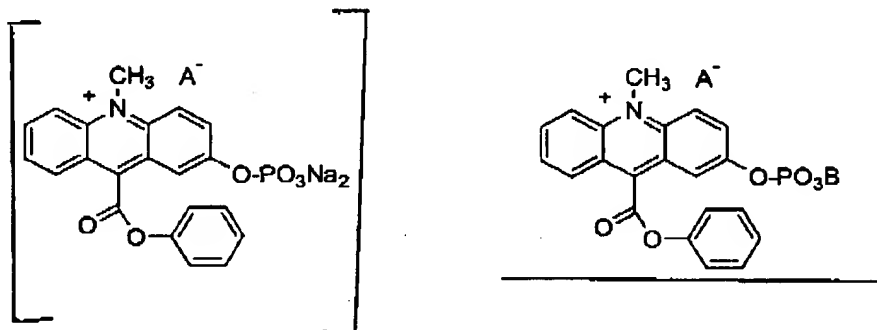
Filed: July 27, 2000

Group Art Unit: 1651

Confirmation No.: 9704

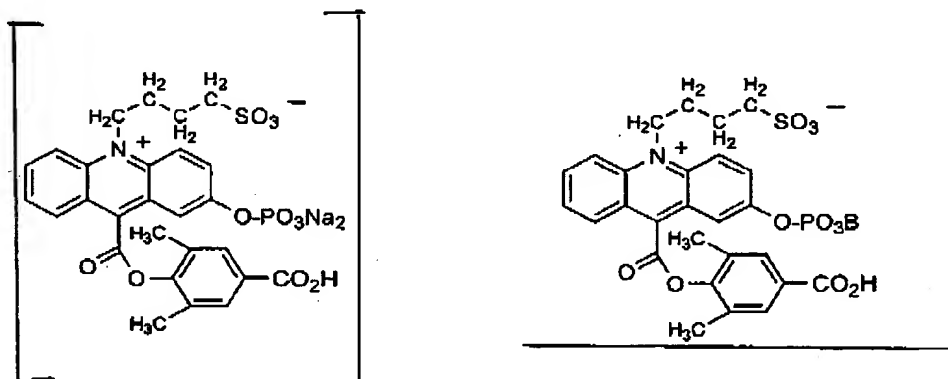
wherein A^- is selected from the group consisting of $CH_3SO_4^-$, FSO_3^- , $CF_3SO_3^-$, $C_4F_9SO_3^-$, $CH_3C_6H_4SO_3^-$, halide, CF_3COO^- , CH_3COO^- and NO_3^- .

53. (Currently amended) The chemiluminescent substrate of claim 43 having the structure



wherein A^- is selected from the group consisting of $CH_3SO_4^-$, FSO_3^- , $CF_3SO_3^-$, $C_4F_9SO_3^-$, $CH_3C_6H_4SO_3^-$, halide, CF_3COO^- , CH_3COO^- and NO_3^- .

54. (Currently amended) The chemiluminescent substrate of claim 43 having the structure



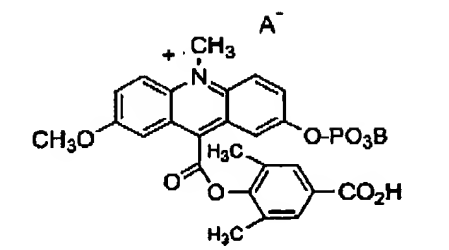
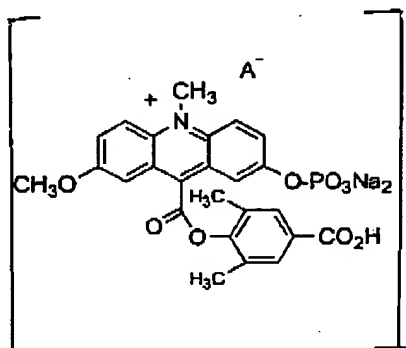
Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651

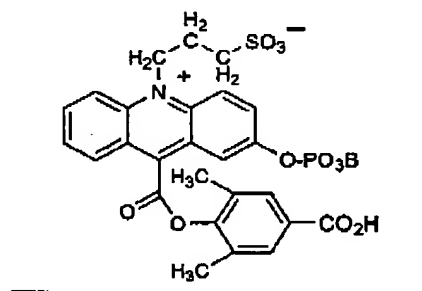
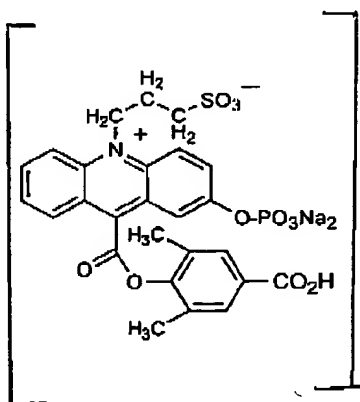
Confirmation No.: 9704

55. (Currently amended) The chemiluminescent substrate of ~~Claim~~
claim 47 having the structure



wherein A^- is selected from the group consisting of $CH_3SO_4^-$, FSO_3^- , $CF_3SO_3^-$, $C_4F_9SO_3^-$, $CH_3C_6H_4SO_3^-$, halide, CF_3COO^- , CH_3COO^- and NO_3^- .

56. (Currently amended) The chemiluminescent substrate of ~~Claim~~
claim 43 having the structure



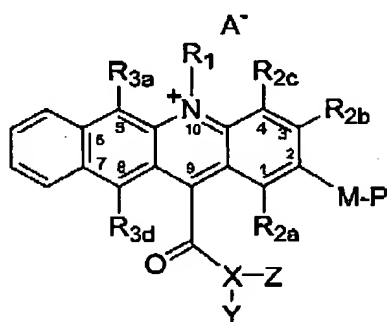
Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651

Confirmation No.: 9704

57. (Currently amended) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



wherein

P is PO_3B or selected from the group consisting of PO_3H_2 , PO_3K_2 , $\text{PO}_3(\text{NH}_4)_2$, PO_3Ca , PO_3Mg , PO_3Na_2 , a sugar moiety and B is a divalent cation or two monovalent cations selected from the group consisting of Na_2 , H_2 , K_2 , Ca and $\text{MgC}(-\text{O})\text{R}$ group wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

R_1 is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

R_{2a} , R_{2b} , R_{2c} , R_{3a} , and R_{3d} can be the same or different, and are selected from a the group consisting of hydrogen, methyl, methoxy, halides, and cyano ($-\text{CN}$);

Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651

Confirmation No.: 9704

A⁻ is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A⁻ not being present if said R₁ substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N ~~or~~ and S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

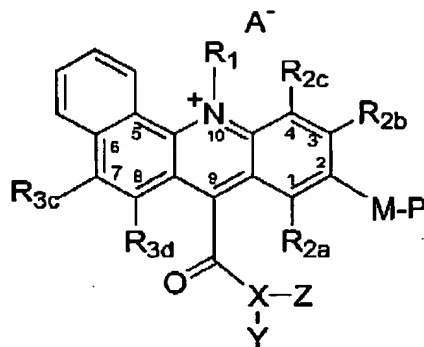
58. (Currently amended) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure

Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651

Confirmation No.: 9704



wherein

P is PO_3B or ~~selected from the group consisting of PO_3H_2 , PO_3K_2 , $\text{PO}_3(\text{NH}_4)_2$, PO_3Ca , PO_3Mg , PO_3Na_2 , a sugar moiety and B is a~~
~~divalent cation or two monovalent cations selected from the group~~
~~consisting of Na_2 , H_2 , K_2 , Ca and MgC(=O)R group wherein R is an~~
~~alkyl group having 1 to 6 carbon atoms;~~

M is oxygen;

R_1 is selected from the group consisting of methyl,
~~sulfoethyl, sulfoethyl, sulfoalkyl, and carboxymethyl;~~

R_{2a} , R_{2b} , R_{2c} , R_{3c} and R_{3d} can be the same or different, and
are selected from a the group consisting of hydrogen, methyl,
methoxy, halides, and cyano ($-\text{CN}$);

A^- is a counter ion for the electroneutrality of the
quaternary nitrogen of the acridinium compounds, said A^- not being
present if said R_1 substituent contains a strongly ionizable group

Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651

Confirmation No.: 9704

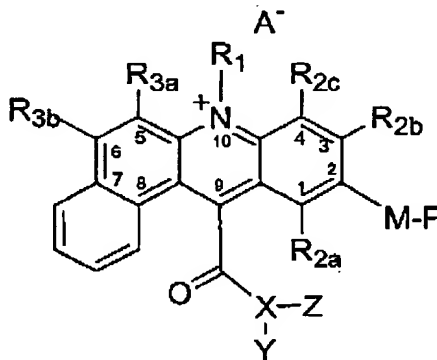
that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N ~~or~~ and S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl and Y is carboxypropyl.

59. (Currently amended) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



-15-

Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651

Confirmation No.: 9704

wherein

P is PO_3B or ~~selected from the group consisting of PO_3H_2 , PO_3K_2 , $\text{PO}_3(\text{NH}_4)_2$, PO_3Ca , PO_3Mg , PO_3Na_2~~ , a sugar moiety and B is a divalent cation or two monovalent cations selected from the group consisting of Na_2 , H_2 , K_2 , Ca and $\text{MgC}(=\text{O})\text{R}$ group wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

R_1 is selected from the group consisting of methyl, ~~sulfopropyl, sulfobutyl, sulfoalkyl,~~ and carboxymethyl;

R_{2a} , R_{2b} , R_{2c} , R_{3a} and R_{3b} can be the same or different, and are selected from a the group consisting of hydrogen, methyl, methoxy, -halides, and cyano ($-\text{CN}$),

A^- is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A^- not being present if said R_1 substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N ~~or~~ and S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-

Application No. 09/626,566

Filed: July 27, 2000

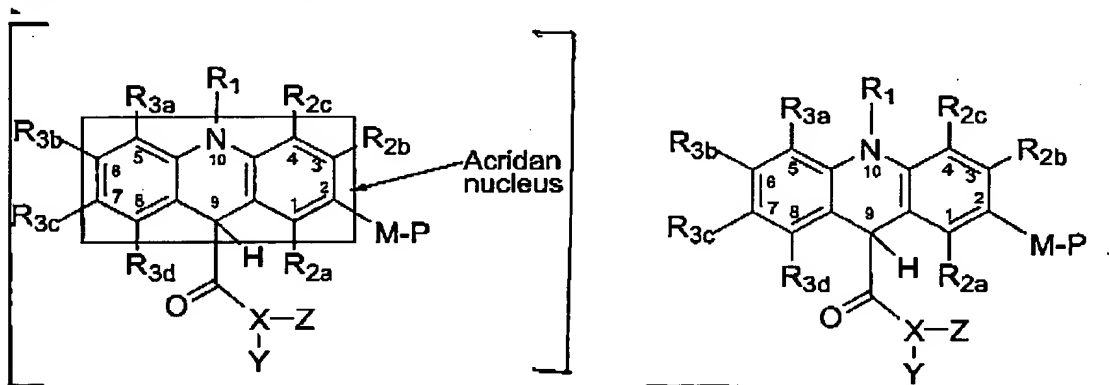
Group Art Unit: 1651

Confirmation No.: 9704

dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl and Y is carboxypropyl.

60. (Currently amended) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



wherein

P is PO_3B or ~~selected from the group consisting of PO_3H_2 , PO_3K_2 , $\text{PO}_3(\text{NH}_4)_2$, PO_3Ca , PO_3Mg , PO_3Na_2~~ , a sugar moiety and B is a divalent cation or two monovalent cations selected from the group consisting of Na_2 , H_2 , K_2 , Ca and $\text{MgC}(-\text{O})\text{R}$ group wherein R is an alkyl group having 1 to 6 carbon atoms;

Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651

Confirmation No.: 9704

M is oxygen;

R₁ is selected from the group consisting of methyl, sulfopropyl, ~~sulfobutyl~~, sulfoalkyl, and carboxymethyl;

R_{2a}, R_{2b}, R_{2c}, R_{3a}, R_{3b}, R_{3c} and R_{3d} can be the same or different, and are selected from ~~a the~~ group consisting of hydrogen, methyl, -methoxy, halides, and cyano (-CN);

A⁻ is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A⁻ not being present if said R₁ substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N ~~or~~ and S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

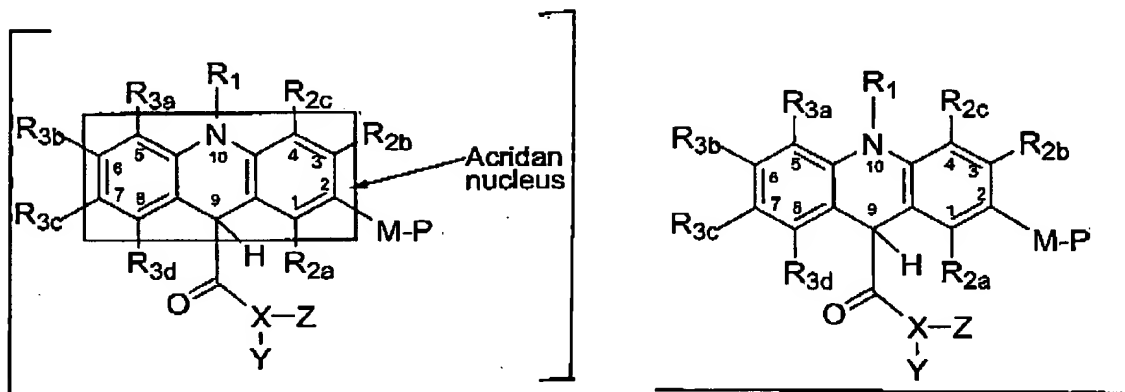
Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651

Confirmation No.: 9704

61. (Currently amended) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



wherein

P is PO_3B or ~~selected from the group consisting of PO_3H_2 , PO_3K_2 , $\text{PO}_3(\text{NH}_4)_2$, PO_3Ca , PO_3Mg , PO_3Na_2~~ , a sugar moiety and B is a divalent cation or two monovalent cations selected from the group consisting of Na_2 , H_2 , K_2 , Ca and $\text{MgC}(-\text{O})\text{R}$ group wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

R_1 is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

R_{2a} , R_{2b} , R_{2c} , R_{3a} , R_{3b} , R_{3c} and R_{3d} can be the same or different, and are selected from the group consisting of hydrogen, methyl, methoxy, halides, and cyano ($-\text{CN}$);

Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651

Confirmation No.: 9704

R₁₁ is selected from the group consisting of hydrogen, -R, substituted or unsubstituted aryl, halides, nitro, sulfonate, sulfate, phosphonate, -CO₂H, -C(O)OR, cyano (-CN), -SCN, -OR, -SR, -SSR, -C(O)R, -C(O)NHR, ethylene glycol and polyethyleneglycol, where R is an alkyl group having 1 to 6 carbon atoms;

A⁻ is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A⁻ not being present if said R₁ substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

X₁ -and X₂ -are the same or different and are selected from the group consisting of O, N ~~or~~ and S, such that,

~~when at least one of X₁ -and X₂ are is O or S, R₁₁ is selected from the group consisting of hydrogen, -R, substituted or unsubstituted aryl, halides, nitro, sulfonate, sulfate, phosphonate, -CO₂H, -C(O)OR, cyano (-CN), -SCN, -OR, -SR, -SSR, -C(O)R, -C(O)NHR, ethylene glycol, or polyethyleneglycol, where R is as defined above; and the corresponding Z₁ -and or Z₂ are is~~
omitted; and

~~when at least one of X₁ -and X₂ is N, the corresponding Z₁ and or Z₂ are is hydrogen, alkyl, aryl or toluenesulfonyl, and R₁₁ is carboxypropyl.~~